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CURRENT LITERATURE

NOTES FOR STUDENTS

Metabolism of fungi.—The problem of the necessity of calcium for fungi has been attacked in different ways by HORI and by Mlle. ROBERT. HORI[1] relied on removing the calcium from his solutions by precipitating it by means of an oxalate. To a solution of "ame" (rice treated with malt extract) in bouillon, he added potassium sulphate and potassium oxalate. In the solution thus obtained *Aspergillus niger*, *A. flavus*, and *Penicillium glaucum* developed well, while *Cephalothecium roseum*, *Rhizopus nigricans*, *Rammularia Citri*, *Botrytis tenella*, *Sclerotinia Libertiana*, and a species of *Entomophthora* developed poorly or not at all. All the fungi developed well in the same solution to which potassium sulphate but no oxalate had been added. From the fact that a number of the fungi did not develop in a solution containing 0.5 per cent of potassium oxalate, the author concludes that these probably require calcium for their development. The conclusion is scarcely justified, since it is not at all probable that calcium is completely removed by potassium oxalate from solutions containing organic substances. The same criticism applies to the experiments of WEIR, which have already been reviewed in this journal.[2]

The problem has been attacked in a more critical manner by Mlle. ROBERT,[3] who worked with *Aspergillus niger*. She showed that the results of the earlier investigators (RAULIN, MOLISCH, LOEW), who came to the general conclusion that calcium was not necessary for the life of most fungi, were due to the fact that they relied on the so-called "pure salts" of commerce. Mlle. ROBERT finds that these "pure salts" always contain calcium. It is thus evident that the conclusion generally reached by those investigators, that calcium is not necessary for the life of fungi, was reached on insufficient grounds. Mlle. ROBERT purified the salts she used until no traces of calcium could be detected in them. In culture solutions made from such purified materials *Aspergillus niger* grows equally well whether calcium is added or not. From dilute solutions containing up to 2.5 mg. per 250 cc. of solution, the calcium is removed almost quantitatively by the fungus and can be recovered in the ash. From stronger solutions containing 10–100 mg. per 250 cc. about 80 per cent of the calcium is removed. When much calcium is absorbed, both the yield and the ash of the fungus are increased, but the increase corresponds to the amount of calcium absorbed. The increase of the ash corresponds to the amount of calcium absorbed calculated as calcium carbonate. It appears from this paper that calcium is of no importance for the development of *Aspergillus niger*, but that when present in the culture medium, it is absorbed and increases the yield and ash only by the amount absorbed.

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[1] HORI, S., Haben die höheren Pilze Kalk nötig? Flora **101**:447, 448. 1910.

[2] BOT. GAZ. **53**:88. 1912.

[3] ROBERT, MLLE., Influence du calcium sur le développement et la composition minérale de l'*Aspergillus niger*. Compt. Rend. **153**:1175–1177. 1911.

In another paper[4] Mlle. ROBERT concludes that the calcium absorbed from solutions by *Aspergillus niger* is quantitatively fixed in the form of calcium oxalate. This conclusion is based on analyses of mycelia from a large number of cultures. Although it was necessary to determine the calcium and the oxalic acid in different cultures, the determinations showed that the oxalic acid present in the mycelium corresponded to the quantity calculated on the assumption that all the calcium fixed was present in the form of calcium oxalate. Owing to the fact that in the cultures without calcium the oxalic acid formed diffuses into the medium, mycelia from such cultures always contain less oxalic acid than those with calcium.

SAUTON,[5] studying the effect of iron on spore-formation of *Aspergillus niger* grown on RAULIN'S solution free from iron, finds that in the absence of iron no spores are formed by this fungus. This fact, while not mentioned specifically by RAULIN, seems nevertheless not to have escaped his notice completely, for he states that in the absence of iron spores become fewer and fewer in proportion to the number of crops of fungus grown on the same solution. When iron is added to cultures free from iron, the yield increases and spores are produced. Cultures with 0.1 mg. of iron per 100 cc. of liquid and those with 1.0 mg. per 100 cc. gave practically the same yield, but spore-formation was almost suppressed in the 0.1 mg. cultures, while those with 1.0 mg. per 100 cc. were black with spores. The cultures without iron made feeble growth and produced no spores. Since there was no difference in growth but a great difference in spore-production between cultures containing 0.1 mg. of iron per 100 cc. of liquid and those containing 1.0 mg. per 100 cc., the author concludes that iron has a specific influence on spore-formation. The possibility that toxic substances are produced in the absence of iron had already been suggested by RAULIN, who noted that in cultures without iron a substance is formed which he regarded as sulphocyanic acid, and FERNBACH had shown that in the presence of ammonium sulphocyanate *Aspergillus niger* grows naturally but does not form spores. SAUTON doubts the formation of sulphocyanic acid, since the liquid from cultures containing 0.1 mg. of iron per 100 cc. (in which spore-formation is almost inhibited) does not give the red color reaction with ferric salts. In cultures without iron, however, a faint red color reaction indicating colorometrically a concentration of 0.005 per cent of sulphocyanic acid could be obtained, but the equivalent of that concentration of ammonium sulphocyanate is without effect on the fungus. That other poisonous substances are formed is doubted by the author, because if to a solution free from iron, on which *Aspergillus* has grown for three days without forming spores, iron is added the culture becomes covered with spores in 24 hours. The suppression of spore-formation is therefore due to the lack of iron and not to a toxic substance. Furthermore, since ferric sulphocyanate also inhibits spore-formation, the favorable effect of iron cannot be due to a possible neutralization of sulphocyanic acid by iron. The author notes a similarity in the behavior of cultures without iron and those suffering from lack of oxygen, and suggests that iron is possibly instrumental in bringing about oxygen fixation.

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[4] ROBERT, MLLE., Mode de fixation du calcium par l'*Aspergillus niger*. Compt. Rend. **154**:1308–1310. 1912.

[5] SAUTON, B., Influence du fer sur la formation des spores de l'*Aspergillus niger*. Compt. Rend. **151**:241–243. 1910.

RAULIN's observation, confirmed by SAUTON, of the occurrence in cultures of *Aspergillus* of a substance giving a red color reaction with ferric salts and therefore regarded as sulphocyanic acid, is of interest in view of the fact that GOSIO in Italy and ALSBERG and BLACK in this country find in cultures of other molds phenol-like substances giving red color reactions with ferric salts.

A later paper by SAUTON[6] consists essentially of a republication of the foregoing paper with some additional observations, although in the meantime the author working with JAVILLIER had published results (reviewed below) leading to an entirely different conclusion from that reached in these two papers. It appears from SAUTON's second paper that the need of iron is not general among fungi, for while *Aspergillus niger*, *A. fumigatus*, *Penicillium glaucum*, and *P. candidum* make only feeble growth on RAULIN's solution free from iron, *Mucor Mucedo*, *Rhizopus nigricans*, and *Racodium coellare* thrive well without iron, or at least with only such minute traces of it that it is impossible to detect them. Since the publication of SAUTON's first paper, BERTRAND and JAVILLIER have called attention to the effect of manganese on the growth of *Aspergillus niger*, without however giving attention to the influence of that element on spore-production. SAUTON finds that the substitution of manganese for iron also stimulates spore-production. He has not been able, however, to obtain sulphate of manganese, which he employed, entirely free from iron, and although the traces of iron thus added were extremely minute, he does not draw any conclusion as to the possibility of substituting one of these elements for the other. Further evidence that the inhibition of spore-production is not due to the formation of sulphocyanic acid was obtained from an experiment in which both sulphur and iron were absent. Although the formation of sulphocyanic acid was not possible under these conditions, spore-production was nevertheless inhibited. On the addition of sulphate of iron spores were formed, showing that it is not sulphocyanic acid which inhibits spore-formation.

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[6] SAUTON, B., Influence du fer sur la culture de quelques moisissures. Ann. Inst. Pasteur **25**:922–928. 1911.

Conclusions very different from the foregoing regarding the special rôle of iron in spore-formation are reached by JAVILLIER and SAUTON[7] in the paper mentioned above. According to this paper iron does not possess a special rôle in the formation of spores, but in the absence of iron, spore-formation is inhibited by the zinc in RAULIN's solution. If both zinc and iron are excluded, spores are readily formed, just as they are when both of these elements are present. If zinc alone is present, spore-formation is inhibited.

The authors suppose that the zinc, which was observed by RAULIN to have a favorable effect in the culture solution proposed by him, exerts toxic effects when the mycelium is poorly nourished through the lack of iron or from some other cause. In the ash of mycelia grown on solutions without iron, no iron was found, although the reactions employed were capable of indicating the presence of 1/1000 of a milligram of the element; hence the authors conclude that if iron is at all essential for spore-formation, the quantity required is so small that it cannot be detected in the ash of the mycelium. It is also stated that the substance giving the red color with ferric salts and supposed by RAULIN to be sulphocyanic acid is not formed when both iron and zinc are absent.

A search for an explanation of the favorable effect, noted by RAULIN and others, of zinc on the growth of *Aspergillus* has led JAVILLIER[8] to investigate the action of that metal on the secretions of invertase by *Aspergillus niger.* The results are suggestive as showing the influence of certain substances on the diffusion of other substances through the protoplasmic membrane. He finds that in cultures free from zinc the liquid does not invert sugar, while culture solutions containing zinc invert sugar readily after *Aspergillus niger* has been grown on them. This difference, however, does not indicate that no invertase is formed in the absence of zinc, for sugar was consumed in both cultures. In cultures free from zinc the enzyme does not diffuse into the liquid.

Several notes have been published by BERTRAND and by BERTRAND and JAVILLIER on the influence of manganese on *Aspergillus niger* and on the relations of this element to zinc and iron when present in the culture medium. Manganese[9] in dilute solutions is found to have a favorable action on the growth of this mold. In solutions containing manganese in concentrations ranging from 1 part in 1,000,000 to 1 part in 100, there was a gradual increase of yield with increasing concentration of the metal. An increasing quantity of the metal was found in the ash, although even in dilute solutions the element was not removed quantitatively, like calcium or zinc. A concentration of 1 part in 50 was deleterious.

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[7] JAVILLIER, M., et SAUTON, B., Le fer est-il indispensable à la formation des conidies de l'*Aspergillus niger?* Compt. Rend. **153**:1177–1180. 1911; also Compt. Rend. Soc. Biol. **71**:589, 590. 1911.

[8] JAVILLIER, M., Influence de la suppression du zinc du milieu de culture de l'*Aspergillus niger* sur la sécrétion de sucrase par cette Mucédinée. Compt. Rend. **154**:383–386. 1912.

[9] BERTRAND, G., et JAVILLIER, M., Influence du manganèse sur le développement de l'*Aspergillus niger.* Compt. Rend. **152**:225–228. 1911; also Bull. Sci. Pharmacologique **18**:65–73. 1911; also Bull. Soc. Chim. France. pp. 212–220. 1912.

Unexpected results[10] showing the sensitivity of plants to extraordinarily minute traces of substances were obtained with manganese in extreme dilutions. After every available means had been used for removing manganese from the ingredients of the culture solutions, it was found that the addition of 1 mg. of manganese in 1,000,000 liters produced a marked increase in yield, while in some cases even the effect of 1 mg. in 10,000,000 liters was noticeable. The effect of manganese in such extreme dilutions is attributed to action of a catalytic nature. The necessity of taking into consideration in physiological experiments the possibility of effects being produced by such minute traces of substances which may be present as impurities in the materials used is pointed out.

The simultaneous addition[11] of zinc and manganese in concentrations at which these metals individually increase the yield of fungus produces a greater yield than the addition of either metal alone. The favorable concentrations were: zinc, 1 part in 100,000 to 1 part in 25,000,000; and manganese, 1 part in 500 to 1 part in 25,000.

To determine if either of these two metals exerted an influence on the absorption of the other, the quantity of manganese absorbed in the presence of zinc was compared[12] with that absorbed from solutions without zinc. The results show that in general the percentage of manganese in the dry weight of the fungus is greater in the presence of zinc. The proportion varies somewhat according to the concentrations of manganese and zinc. The effect of the zinc is more marked in dilute solutions and disappears in more concentrated solutions.

To manganese is attributed a special rôle in spore-formation formerly ascribed by RAULIN and by SAUTON to iron. The effect noted by these investigators BERTRAND[13] believes to be due to the introduction of minute traces of manganese from which it is difficult to free some of the other salts used in RAULIN's solution. He finds that when *Aspergillus* is grown in a solution free from manganese, but containing both zinc and iron (1 part in 100,000) no spores are produced. If a trace of manganese is added, the surface becomes black with conidia.

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[10] BERTRAND, G., Extraordinaire sensibilité de l'*Aspergillus niger* vis-à-vis du manganèse. Compt. Rend. **154**:616–618. 1912.

[11] BERTRAND, G., et JAVILLIER, M., Influence combinée du zinc et du manganèse sur le développement de l'*Aspergillus niger*. Compt. Rend. **152**:900–902. 1911; also Bull. Sci. Pharmacologique **18**:321–327. 1911.

[12] ———, Influence du zinc et du manganèse sur la composition minerale de l'*Aspergillus niger*. Compt. Rend. **152**:1337–1340. 1911.

[13] BERTRAND G., Sur le rôle capitol du manganèse dans la formation des conidies de l'*Aspergillus niger*. Compt. Rend. **154**:381–383. 1912.

A later paper by BERTRAND and JAVILLIER[14] consists of recapitulation of the data in the foregoing papers by them, together with a general discussion of the results obtained.

The availability of various nitrogenous compounds as sources of nitrogen for fungi has been investigated by BRENNER, by RITTER, and by KOSSOWIG. BRENNER[15] in a brief paper gives the general results of an investigation designed to determine the relative value of various nitrogenous compounds for the nutrition of *Aspergillus niger*. The compounds were given in a concentration equivalent in nitrogen to a 0.5 per cent ammonium chloride solution. The nutritive value of the substances was determined by ascertaining the time required for the cultures to attain a maximum weight on a given substance. Both the time required for reaching a maximum development and the weight of fungous substance produced were taken into consideration. By analysis of the fungus crops and the residual culture medium, the qualitative and quantitative interchange of nitrogen between the fungus and the medium was determined. The results showed that a large number of compounds are not suitable sources of nitrogen. Of this category are free ammonia, sodium nitrite, ammonium valerianate, and potassium cyanide, all of which are poisonous, and tetramethylammonium chloride, nitroguanidin, nitromethane, isoamylacetate, pyridin chloride, and piperidine chloride, which are not assimilable. The order of nutrient value of the other compounds tried is somewhat as follows: first, ammonium lactate, ammonium tartrate, asparagin, ammonium succinate, and ammonium oxalate; second, the ammonium salts of sulphuric, hydrochloric, nitric, and phosphoric acids, and carbamid; third, ammonium acetate, ammonium formate, nitrosodimethylamin chloride, sodium nitrate, pyridin nitrate, normal and isobutylamin chloride, guanidin nitrate, and guanidin chloride. Of less nutritive value are isoamylamin chloride, hydroxylamin sulphate, benzylamin sulphate, dicyandiamid, and perhaps acetonitril.

A study of the composition of the fungus and the changes in the medium showed that after a period of growth of about 4 days, processes of degeneration began in parts of the fungus. These were accompanied by the secretion of nitrogen as ammonia or as organic nitrogenous compounds. As a rule, regardless of the nature of the compounds, about one-half of the nitrogen present in a solution containing the nitrogen equivalent of a 0.5 per cent ammonium chlorid solution was taken up by the first crop of fungus grown. Subsequent crops having less nitrogen at their disposal contained a lower percentage of nitrogen than the first crops on the same solution. No general conclusions were deducible from a quantitative study of the residual culture fluid.

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[14] BERTRAND, G., et JAVILLIER, M., Action du manganèse sur le développement de l'*Aspergillus niger*. Ann. Inst. Past. **26**: 241–249. 1912.

[15] BRENNER, W., Untersuchungen über die Stickstoffernährung des *Aspergillus niger* und deren Verwertung. Ber. Deutsch. Bot. Gesells. **29**: 479–483. 1911.

RITTER[16] in confirmation of results published in a former paper emphasizes the fact that *Aspergillus glaucus*, *Cladosporium herbarum*, and *Mucor racemosus*, known as "nitrate fungi" on account of their supposed preference for nitrogen from the nitrate ion, thrive equally well or better on ammonium salts. The slight alkalinity arising in cultures with potassium nitrate is, according to him, not the cause of the depression of growth in such cultures. The assimilation of nitrogen from nitrates takes place by means of a reduction of the nitrates to nitrites, as in bacteria and the higher plants. Since nitrites are not stable in acid solutions, it is necessary to keep the cultures neutral or alkaline to show the formation of nitrites. In such cultures nitrites resulting from the reduction of nitrates were shown to occur.

KOSSOWICZ[17] examined a number of fungi with reference to their ability to obtain nitrogen from calcium nitrid supplied in a nutrient solution containing per liter 2.5 gms. dipotassium hydrogen phosphate, 0.5 gms. magnesium sulphate, 25 gms. cane sugar, and 5 gms. tartaric acid. The calcium nitrid was added after sterilization. The fungi used were *Botrytis bassiana, Penicillium crustaceum, P. brevicaule, Mucor Boidin, Cladosporium herbarum, Phytophthora infestans, Aspergillus glaucus, A. niger, Isaria farinosa,* and a species of *Fusisporium.* Of these only *Phytophthora infestans, Botrytis bassiana,* and *Mucor Boidin* showed any growth after three months. *Phytophthora infestans* caused the production of ammonia in the culture medium, but in the cultures of the other two fungi ammonia could not be detected with Nessler's reagent. The poisonous effect of calcium nitrid was shown by the feeble growth of the foregoing fungi on solutions containing calcium nitrid and ammonium chloride compared with their growth on solutions containing ammonium chloride alone as a source of nitrogen.

The availability of different phosphorus compounds as sources of phosphorus for *Aspergillus niger* has been studied by DOX.[18] Of the inorganic compounds he finds that orthophosphate, pyrophosphate, and metaphosphate serve well as sources of phosphorus, while hypophosphite and phosphite containing trivalent phosphorus, while not toxic, do not supply phosphorus in an available form. Several organic substances containing phosphorus were found to be excellent sources of phosphorus for the mold. The substances tested were phytin, sodium glycerinophosphate, sodium nucleinate, lecithin, casein, and ovovitellin. The author suggests that in all these cases phosphoric acid is first split off from the organic compound by means of enzymes. This suggestion is strengthened by the facts that IVANOFF has prepared an enzyme from this mold which decomposes nucleic acid with the liberation of phosphoric acid, and the author himself has shown the presence in another mold of an enyzme capable of hydrolyzing casein.—H. HASSELBRING.

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[16] RITTER, G. E., Ammoniak und Nitrate als Stickstoffquelle für Schimmelpilze. Ber. Deutsch. Bot. Gesells. **29**:570–577. 1911.

[17] KOSSOWICZ, A., Über das Verhalten einiger Schimmelpilze zu Kalkstickstoff. Zeitschr. Gärungsphysiologie **1**:124, 125. 1912.

[18] DOX, A. W., The phosphorus assimilation of *Aspergillus niger.* Journ. Biol. Chem. **10**:77–80. 1911.

Current taxonomic literature.—O. AMES (Phil. Journn. Sci. Bot. **7**:1–27. 1912) in continuation of his studies in the Orchidaceae has published 27 new species of orchids from the Philippine Islands.—O. BECCARI (Pomono Coll. Journ. Ec. Bot. **2**:253–276. 1912) under the title "Palms indigenous to Cuba I" recognizes 14 genera, 3 of which are treated in detail in the present paper. One new species is added to the genus *Oreodoxa*, namely *O. princeps*. —C. H. BISSELL and M. L. FERNALD (Rhodora **14**:91, 92. 1912) record a new variety of *Lespedeza* (*L. capitata* var. *stenophylla*) from Illinois and Connecticut. G. BITTER (Rep. Sp. Nov. **10**:489–501. 1912) places on record supplementary data to his recent monograph of the genus *Acaena* and includes descriptions of new varieties from Central and South America. The same author (*ibid.* 529–565) under the title "Solana nova vel minus cognita I" has published 36 new species of *Solanum* also from Central and South America.—S. F. BLAKE (Rhodora **14**:102–106. *pl. 94*. 1912) characterizes several new forms of *Peltandra virginica*.—E. BRAINERD (Bull. Torr. Bot. Club **39**:85–97. *pls. 5–7*. 1912) presents an interesting article on "Violet hybrids between species of the *palmata* group," recording several new hybrids in the genus.—A. BRAND (Univ. Calif. Pub. Bot. **4**:209–227. 1912) presents a preliminary consideration of the Hydrophyllaceae of the Sierra Nevada region. Several new varieties and forms are described and certain changes in nomenclature are made. The same author (Phil. Journ. Sci. Bot. **7**:29–36. 1912) records the results of further investigations on the Symplocaceae of the Philippine Islands, adds 3 new species to *Symplocos*, and gives a revised key to the 26 recognized Philippine species of this genus.—T. S. BRANDEGEE (Univ. Calif. Pub. Bot. **4**:269–281. 1912) under the title "Plantae Mexicanae Purpusianae IV" has published 29 new species and 2 varieties of flowering plants. One new genus (*Oxyrhynchus*) of the Leguminosae is proposed.—H. BRAUNS (Verhandl. K. K. Zoolog.-Bot. Gesell. Wien **61**:139–143. 1911) describes 6 new species of *Nitella*, 2 of which are from Costa Rica.—J. BROADHURST (Bull. Torr. Bot. Club **39**:257–278. *pls. 21, 22*. 1912) under the title "The genus *Struthiopteris* and its representatives in North America" gives a revision of the genus, recognizing 9 species, of which 2 are new to science.—J. BURTT-DAVY and R. POTT-LEENDERTZ (Ann. Transvaal Mus. **3**:119–182. 1912) have issued a check list of the flowering plants and ferns of the Transvaal and Swaziland. Approximately 3240 species are enumerated, representing 920 genera and 157 families.—E. J. BUTLER (Ann. Botany **25**:1023–1035. 1911) describes and illustrates a new genus and species (*Allomyces arbuscula*) of the Leptomitaceae from India.—E. CLAASSEN (Ohio Nat. **12**:543–548. 1912) records about 125 species and varieties of lichens from